HEMOTROPIC DISEASES
OF THE
DOG, CAT, AND HORSE

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CANINE EHRLICHIOSIS

Pathogenesis, Clinical Diagnosis, and Treatment

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ETIOLOGY

Four genera of *Ehrlichia* are now known to be pathogenic in the dog. *Ehrlichia canis*, prototype of the genus and the cause of canine ehrlichiosis, infects canine mononuclear cells. *Ehrlichia platys* infects canine platelets. Clinical signs due to natural *E. platys* infection are rare unless concurrent infection with another blood-borne organism is present. *Ehrlichia equi* predominantly infects and produces severe disease in the horse but can infect canine granulocytes and cause mild signs of illness. Most recently, it has been determined that natural exposure to *E. risticii*, causative agent of equine monocytic ehrlichiosis (Potomac horse fever), can induce a mild to moderate clinical disease in dogs. This paper will primarily address canine ehrlichiosis caused by *E. canis*.

Canine ehrlichiosis is a tick-borne rickettsial blood disease of domestic and wild canids. The disease may be presented as acute, subclinical, chronic, or severe chronic in form. The latter form is often referred to as tropical canine pancytopenia (TCP). The etiologic agent of canine ehrlichiosis is *Ehrlichia canis*, which is classified within the tribe Ehrlichieae, family Rickettsiaceae, order Rickettsiales.

Ehrlichia canis is a gram-negative, small coccoid, ellipsoid, or often pleomorphic microorganism which occurs within intracytoplasmic vacuoles of monocytes and lymphocytes. The agent may occur singly (elementary bodies) or in the form of small clusters (initial bodies) or large, tightly-packed clusters (morulae). The size of elementary bodies is in the range 0.2 to 0.4 μm, initial bodies from 0.5 to 4 μm, and morulae from 3 to 6 μm.

EPIZOOTIOLOGY

The brown dog tick, *Rhipicephalus sanguineus*, is the sole biologic vector involved in the transmission of *E. canis*. Transstadial but not transovarial transmission has been demonstrated in the tick. The microorganism may also be transmitted from infected to susceptible dogs by whole blood

inoculation. Accordingly, potential blood donor dogs should be confirmed free of *E. canis* infection prior to their use. Canine ehrlichiosis has been diagnosed worldwide wherever the vector tick exists. The majority of cases in the United States have been reported to occur in the western, southwestern, and southeastern regions, as well as Hawaii. The disease may often be diagnosed in regions where the brown dog tick is not found; however, case histories usually reveal that such infections were imported from enzootic areas.

CLINICAL SIGNS

Canine ehrlichiosis is a progressive disease. The onset of the acute phase of the disease generally occurs within 8 to 16 days following the bite of an infected tick or after inoculation of infected blood. This stage lasts 2 to 4 weeks and is usually characterized by fever (104° to 106°F), depression, anorexia, serous to purulent oculonasal discharge, lymphadenopathy, and occasionally corneal opacity. Edema of the limbs and ataxia may also be present. During this phase, the organism multiplies by infecting mononuclear cells which pass to tissues of the mononuclear-phagocyte system. The acute stage is generally followed by a subclinical phase of varying duration. During this period, the dog may appear clinically normal; however, in experimental situations, all blood values remain at subnormal levels when compared with values recorded prior to infection. The disease may remain at a mild, chronic level with occasional episodes of fever, or develop into the severe chronic phase known as TCP. The development of this severe chronic phase is dependent upon the inability of the host to mount a sufficient immune response to maintain balance with the parasite. The factors which determine each animal's ability to mount a sufficient immune response and the clinical severity of the disease are not known for certain but probably include strain variation, host age and breed, physical or immunologic stress, and the presence of concomitant disease. Breed susceptibility is particularly important in German shepherd dogs.

They appear to have depressed cell-mediated immunity as compared to other breeds and are more likely to develop severe disease.

Typically, TCP is characterized by fever, corneal opacity, hyphema, regenerative or nonregenerative anemia, and a severe leukopenia and thrombocytopenia. During the early phase of the disease, bone marrow hyperplasia is evident. However, as the disease progresses, cellular elements in the marrow become exhausted, producing a state of hypoplasia. Owing to the severe thrombocytopenia, epistaxis, petechiation of mucous membranes, hemorrhagic skin lesions, and peripheral edema are often observed. Mononuclear infiltration and hemorrhage of the meninges may result in an arched back, cervical or back pain, ataxia, paresis, and cranial nerve deficits. Death during this phase of the disease is generally due either to extensive mucosal and serosal hemorrhages or to secondary bacterial infections prompted by the dog's debilitated condition.

CLINICAL PATHOLOGY

The most prominent clinicopathologic manifestations of ehrlichiosis are a greatly increased erythrocyte sedimentation rate, thrombocytopenia, and a slight to severe pancytopenia. In recovering animals, there is a gradual to spontaneous improvement in thrombocyte levels. The appearance of bone marrow specimens obtained by aspiration cytology during the acute phase of the disease may range from normal cellularity to various degrees of hypocellularity.

Affected dogs may develop severe chronic ehrlichiosis subsequent to surgery or chemotherapy. Consequently, the severe chronic phase of ehrlichiosis may be precipitated by stress or by immunosuppression after a prolonged subclinical infection.

Hypergammaglobulinemia that develops during the acute febrile phase persists during the subclinical and subsequent terminal phase of the disease. These excessive γ-globulins, however, are not all specific for *E. canis*. Such manifestations are often misdiagnosed and attributed to an autoimmune process rather than to canine

ehrlichiosis. Other biochemical findings include an increase in hepatic enzyme activity (serum alanine aminotransferase or SALT and alkaline phosphatase), particularly during the acute phase. Bone marrow cytology depends upon the stage of the disease. Pancytopenia is more likely with chronic ehrlichiosis. In contrast to the acute stage, bone marrow examination usually reveals moderate to marked hypocellularity with variable degrees of plasmacytosis.

Among various prominent pathologic manifestations of fatal ehrlichiosis are extensive plasmacytosis and perivascular cuffing in most parenchymal organs, particularly the lung, meninges, kidneys, and spleen, suggesting an immunopathologic phenomenon. Such a phenomenon has been further substantiated by the finding that lymphocytes from infected dogs exert a cytotoxic effect upon autologous monocytes. This monocytotoxicity was shown to bear a temporal relationship to the thrombocytopenia. An indication that the thrombocytopenia in canine ehrlichiosis is immunologically mediated has been provided by evidence that serum from diseased dogs inhibits platelet migration *in vitro*. Scanning electron microscopy has indicated that the platelet migration inhibition factor (PMIF) interferes with platelet migration by inhibiting platelet pseudopod formation. Affected platelets become rounded and show evidence of clumping and leakage. Necropsy findings vary depending upon the stage of disease. Weight loss and emaciation are usually present in chronic cases. Petechial and ecchymotic hemorrhages may be present in the skin, tissue, and visceral surfaces of many organs. Lymphadenopathy, splenomegaly, and hepatomegaly are frequently present, particularly during the acute phase.

DIAGNOSIS

Diagnosis of acute ehrlichiosis by microscopic detection of the organism in Giemsa-stained blood smears or buffy coat preparations is generally difficult, if not impossible, because the percentage of infected monocytes within the peripheral blood is extremely low.

An indirect fluorescent antibody (IFA) test, using *in vitro* culture-derived organisms as antigen, is currently the only available specific means for confirmatory diagnosis of canine ehrlichiosis by detection and titration of antibodies to *E. canis*. In experimentally infected dogs, antibodies are usually first detectable between days 7 and 14 postinfection and 100% detectable by day 21. This variation is apparently due to individual animal responses rather than the volume of infectious inoculum. The serum titer in the IFA test may vary from 1:10 to 1:10,240 or greater, depending on the stage of infection, immune involvement of individual dogs with the agent, and breed of the dog. During the acute stage of infection, there is generally a very rapid increase in titer which, following recovery, is maintained at varying levels. Dogs in the chronic phase of ehrlichiosis generally reveal titers of 1:81,920 or greater. These extremely elevated titers generally indicate that the dog has been infected for a considerable length of time.

The presence of concurrent disease in a dog with ehrlichiosis can complicate the diagnosis and prognosis. Concurrent disease with babesiosis, hepatozoonosis, or hemobartonellosis are not too uncommon and suggest that more than one disease may be transmitted simultaneously by the vector tick infected with multiple organisms. In addition, existing autoimmune syndromes may mask various clinical signs associated with ehrlichiosis.

THERAPY

Specific Therapy

Tetracycline remains as the primary treatment of choice for ehrlichiosis. The recommended therapeutic regimen consists of tetracycline hydrochloride or oxytetracycline (preferred) given orally at 33 mg/lb divided t.i.d. for 2 to 4 weeks. While in many cases improvement may be noticed within 1 to 2 days, patient response may vary. A return to the afebrile state and an improvement in attitude are usually the first signs of clinical improvement. However, dogs in the chronic stage of disease usually

take longer to respond. Dogs with chronic and particularly severe thrombocytopenia have a guarded prognosis and may show little to no response.

Doxycycline is a semisynthetic tetracycline analogue that is very lipid soluble. As such, it readily penetrates cells and, therefore, may be more effective in the elimination of *Ehrlichia* organisms. Treatment for 14 to 21 days at a dosage rate of 5 to 10 mg/kg orally is usually most effective in the treatment of chronic ehrlichiosis. The treatment time can be reduced to 5-7 days with intravenous administration.

If this treatment is effective in totally eliminating the organism from the affected dog, the IFA titer should gradually subside to a minimal or nondetectable level within 6 to 18 months posttreatment. Significant titer changes in chronically infected dogs may take longer. Periodic serologic screening and clinical follow-ups are recommended. If there is a significant increase in antibody titers and/or clinical abnormalities noted (including asymptomatic dogs), treatment should be repeated.

Chloramphenicol has been used to treat ehrlichiosis. However, because its efficacy is less than the tetracyclines, and the drug can directly interfere with heme synthesis, its use is not recommended.

Imidocarb dipropionate is a diamidine antiprotozoal drug which is effective for the treatment of a variety of animal protozoal diseases. Imidocarb is given as a single intramuscular injection (5 mg/kg). Imidocarb is the drug of choice for concurrent *Babesia* infections since the drug is particularly effective against canine babesiosis. Concurrent infections with both ehrlichiosis and *Babesia* reportedly can be effectively treated with two intramuscular doses of imidocarb given 14 days apart.

Supportive Therapy

Dehydration should be corrected with the administration of polyionic fluids. Food and water intake is particularly important to animals with chronic debilitating disease.

Blood transfusions are indicated for massive hemorrhage or chronic anemia that has become life threatening. Vasoconstrictive astringents (epinephrine or phenylephrine) may be useful for resolution of a nasal hemorrhage. Nutritional support is important for recovery of the hemopoietic system. B vitamins (particularly B12) and folic acid supplementation are indicated. Iron supplementation is also indicated in animals with chronic blood loss anemia.

Vincristine has also been used to treat thrombocytopenia induced by *Ehrlichia* as well as other illnesses. It can be given intravenously at weekly intervals (0.01 to 0.025 mg/kg) or as vincristine-loaded platelets.

PREVENTION

Since transmission by vectors appears to be the only means by which the disease can be naturally transmitted to other animals, tick control is the best means of preventing the disease. Environments should be sprayed and animals dipped every 1 to 2 weeks in endemic areas. Incidences in kennels can be reduced by testing all animals and treating those with positive titers prior to introduction into the kennel. New additions should be quarantined until tested negative and treated if positive.

The only preventive therapeutic measure for canine ehrlichiosis thus far available is administration on a continuous basis of tetracycline at a low dosage (6.6 mg/kg/day). This may be most highly effective for dogs traveling to enzootic areas for a limited period.

EHRLICHIA CANIS SEROLOGY

The specimen required is 1.0 ml serum (RTT or STT). The test is an IFA, and positive results are titered out completely to end point.

INTERPRETATION OF TEST RESULTS

Intracellular inclusions of E. canis in peripheral blood smears may occasionally be seen, but their persistence in peripheral blood is brief. Therefore, serologic testing is the best means of detecting the disease. Antibody response to Ehrlichia canis is first detectable at 7-21 days after infection and continues to rise in untreated dogs. The IFA titer decreases gradually after the parasite is eliminated by treatment, but it may persist indefinitely, especially in chronic cases. In such cases where dogs remain seropositive following successful treatment of clinical illness, it is strongly recommended that, aside from follow-up serology, hematologic parameters (CBC, particularly platelets) be evaluated on a routine basis (every 4-6 months) to confirm that the dog has been successfully cleared of infection since subclinical carriers may relapse at any time and/or serve as a source of infection through the tick for susceptible dogs.

High Titer (>1:20,480)

Because the serum is titered out completely (i.e., diluted until a positive reaction is not longer detected), some very high titers are reported. Titers >1:20,480 generally indicate that the dog is in the chronic phase of the disease and was exposed some time ago (up to several years ago). If the owners are unaware of any episode of clinical disease in the past, the dog probably experienced a very mild acute phase of the disease. If the dog was ill and was treated, the titer may still remain high even though the dog is no longer sick. In some chronic cases with high titers, the titers may go down slightly over 6 months to 1 year. Periodic checkups for signs of disease, including a CBC, are recommended for dogs with persistent titers (every 3-4 months for dogs with very high titers). Any abnormalities detected on the CBC (e.g. a WBC and/or platelet count which is below the normal values), indicates that the dog was not cleared of infection and should be treated as described below.

Moderate Titers (1:160 - 1:10,240

A moderate titer indicates that the dog is in the late acute/early convalescent phase of disease and that exposure was probably relatively recent. When such dogs are treated, their titers tend to decrease over a shorter period of time (approximately 3-6 months). Generally, if the illness has been brief, they may convert completely to a negative status over a 6-month period after treatment. However, some dogs have been known to remain seropositive, albeit at a significantly lower titer, for an indefinite period of time.

Low Titers (1:40 - 1:80)

Dogs with low titers are most likely in the early acute phase of ehrlichiosis or received a mild exposure but do not have an active infection presently. In such cases, a follow-up sample (1-2 weeks) is strongly recommended. If the antibody levels do not increase, such low titers may represent an inactive (subclinical) infection or a cross-reaction with other variants of Ehrlichia. Increasing antibody levels would suggest an active infection. Seroconversion to a negative status usually occurs within a few months following treatment.

Very Low Titers (1:10 - 1:20)

Animals with very low titers should have a second serum sample submitted in 1-2 weeks. If no increase in antibody titer is detected, the dog most likely received a very mild exposure at some point in time and is not actively infected with E. canis.

ADDITIONAL COMMENTS

Hyperglobulinemia generally is present in dogs affected by canine ehrlichiosis. In some dogs experiencing the severe chronic phase of ehrlichiosis, bone marrow hypoplasia may occur, resulting in severe hypoglobulinemia with a concomitant decrease in serum antibody titers. Thus, serology results should be interpreted in conjunction with clinical presentation, hematology and biochemistry in order to establish a definitive diagnosis of ehrlichiosis.

TREATMENT RECOMMENDATIONS

Any dog seropositive for *E. canis* should be treated accordingly. The standard treatment for ehrlichiosis in dogs is tetracycline at 11 mg/lb t.i.d. for 3-4 weeks. However, some researchers suggest that more satisfactory results will be obtained with one of the following regimens:

Oxytetracycline at 11 mg/lb t.i.d. for a minimum of 21 days

Doxycycline at 5-10 mg/kg (divided b.i.d.) for 14-30 days

(Note: It is recommended that dogs which demonstrate a chronic infection be administered 8-10 mg/kg doxycycline for 21-30 days.)

CANINE BABESIOSIS

ETIOLOGY

Canine babesiosis is a hemoprotozoan infection of dogs residing in many tropical, semi-tropical and semi-arid regions of the world. Both *Babesia canis* and *B. gibsoni* are known to infect dogs in such regions of the U.S., although the latter is less common.

Babesia parasites may be observed within host erythrocytes, particularly during the acute stage of infection. *Babesia canis*, the larger of the two species, is a pyriform, teardrop- or pear-shaped, protozoal organism which measures 5-7 μm in length by 2-4 μm in width. While the organisms frequently occur in pairs, up to 8 organisms have been noted within a single erythrocyte. *Babesia gibsoni* is smaller than *B. canis*, measuring 1-3 μm in diameter and is observed most often within erythrocytes as single ring-form parasites.

EPIZOOTIOLOGY

In the U.S., the primary vector for transmission of *B. canis* or *B. gibsoni* is the brown dog tick, *Rhipicephalus sanguineus*. Since this tick is also the vector for *Ehrlichia canis*, dual infections are not uncommon, particularly in the southwest region of the U.S. While *B. canis* infections in dogs are most often recognized in the southwest and southeast regions of the U.S., *B. gibsoni* infections appear to be more geographically limited at this time. This species of Babesia was originally more prevalent in southeast Asia, however, over the past few years various foci of canine babesiosis caused *B. gibsoni* have become established in the U.S. While a few cases have been confirmed in the southwestern, southeastern and northeastern regions of the U.S., the majority of cases have been detected in dogs residing in southern California, particularly in the counties of San Bernardino, Kern and Los Angeles.

Aside from the tick, transmission of *Babesia* infections may also occur through blood transfusions from infected to susceptible dogs and by contaminated needles and instruments. Certain biting insects may also serve as mechanical vectors. Transplacental transmission of *Babesia* infections can occur. However, puppies infected *in utero* are less susceptible to clinical disease due to passive maternal immunity but remain carriers of the disease if untreated. Infections are also less severe in dogs which become infected as adults and most severe in those which become infected as older puppies and young (< 2 years) adult dogs.

Clinical Signs and Pathogenesis

Clinical signs of canine babesiosis may vary greatly, depending on the stage of disease, age and immune status of the dog and complications from concurrent infections. Basically, there are four stages of canine babesiosis which may be recognized. The acute phase of babesiosis is most often characterized by fever, lethargy, anorexia, splenomegaly, hepatomegaly, lymphadenopathy, diarrhea or constipation, vomiting and congestion of mucous membranes which may become pale or white, hematuria and/or hemoglobinuria. Dogs may also become icteric and develop mild to severe ascites. CNS signs, muscular aberrations, respiratory symptoms and circulatory disturbances are also common findings during the acute stage. Clinical pathology usually reveals hemolytic anemia that is regenerative, bilirubinemia, bilirubinuria, varying degrees of hemolysis and lymphocytosis. Thrombocytopenia may occur in both mild and severe infections with *B. canis* but is usually more marked in severe cases. Mechanisms responsible for the thrombocytopenia are not clearly understood, however, it appears to develop independent of DIC, which is also a complication of severe canine babesiosis. The acute form of canine babesiosis is most commonly observed in puppies and young dogs.

The peracute phase of the disease is most often represented by a very short history of illness. There is an extreme sudden onset of clinical signs followed by irreversible shock and death. Severe

neurologic manifestations are due to sludging of parasitized erythrocytes within the capillaries of the CNS. Hypoxia and DIC are common findings in the peracute phase of babesiosis. This phase is most common in heavily tick-infested young dogs.

The chronic form of canine babesiosis is characterized by intermittent fever, capricious appetite and marked loss in body condition. However, mild anemia and icterus may be the only notable signs. Concurrent infections with *E. canis* are common, thereby complexing the recognition of chronic babesiosis.

The subclinical carrier phase is the most common form of babesiosis in adult dogs in the U.S. The carrier results from a fine equilibrium between the parasite and the immune mechanisms of the host. A breakdown in homeostasis resulting in clinical babesiosis may develop following stress, immunosuppression due to chemotherapy or other factors, or splenectomy. Such dogs may serve as an important source of infection if used for blood donors or for breeding purposes.

DIAGNOSIS

Examination of the feathered edge of Giemsa-stained blood smears during the acute stage of infection may assist in the early diagnosis of canine babesiosis by revealing the presence of Babesia-infected erythrocytes. Since infected erythrocytes have a lower specific gravity than non-infected cells, blood collected from peripheral ear tip capillaries or the nail bed is preferred. The percent of parasitized erythrocytes may vary greatly (< 1% - 50%) but is of no prognostic value. *Babesia canis* may be differentiated from *B. gibsoni* based on size of the parasite (2.5 μm x 5.0 μm vs. 1.0 μm x 3.2μm) and configuration within the host cell (paired vs. single).

Serology provides a highly accurate and reliable method for the detection of late acute, chronic or subclinical infections. While several serologic assays have been developed, the indirect fluorescent antibody (IFA) test appears to be the most specific and sensitive method available. While there is some reference in the

literature to serologic cross-reactivity between *B. canis and B. gibsoni*, it is believed by the author that the antigen source for such tests may have involved a dual infection with both species. Comprehensive studies of *B. canis and B. gibsoni* isolates utilized by at least two laboratories (ProtaTek Reference Laboratory and the University of California-Davis) have failed to reveal any serologic cross-reactivity between the two species, thereby allowing for the detection of specific antibodies and confirming the *Babesia* species involved in the infection. Antibody titers of 1:40 are considered suggestive of infection. Antibody titers ≥ 1:80 are considered as positive for active infection. It is recommended that all blood donors, particularly Greyhounds, due to a number of outbreaks of babesiosis affecting various groups within this breed, be serologically screened prior to their entry into the donor program. Any dog found seropositive (≥ 1:40) should not be used as a blood donor. All untreated, serologically positive dogs should be considered carriers of the disease.

TREATMENT

Several drugs have been determined to be effective against canine babesiosis. *Babesia canis* is more readily treatable than *B. gibsoni*. Clinical relapses are not uncommon following treatment for infections caused by the latter agent. Diminazine aceturate (Berenil, Hoechst; Ganaseg, Squibb) is used to treat both *B. canis* and *B. gibsoni* infections. The dosage consists of a single IM injection at 3.5 mg/kg. Imidocarb diproprionate (Imizol, Wellcome), when given in one single injection or two injections one week apart, at a dosage of 5 mg/kg IM is highly effective against infections caused by *B. canis* only. Phenamidine (Lomadine, May and Baker) given twice at 15 mg/kg SC on 2 consecutive days has also been found effective against infections caused by either *B. canis* or *B. gibsoni*.

Supportive therapy, particularly for those dogs which have severe anemia, is very important and should include, as deemed appropriate, blood transfusions, hematinics and electrolyte

solutions. Lactated Ringer's solution is recommended for those dogs which become dehydrated.